(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 1 164 136 A1

(12) EUROPEAN PATENT APPLICATION

(43) Date of publication:
19.12.2001 Bulletin 2001/51

(21) Application number: 01108984.4

(22) Date of filing: 11.04.2001

(51) Int Cl.[7]: C07D 487/22, C07D 471/22, A61K 41/00, A61P 35/00 // (C07D487/22, 257:00, 209:00, 209:00, 209:00, 209:00), (C07D471/22, 257:00, 221:00, 209:00, 209:00, 209:00, 209:00)

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE TR
Designated Extension States:
AL LT LV MK RO SI

(30) Priority: 12.06.2000 US 592150

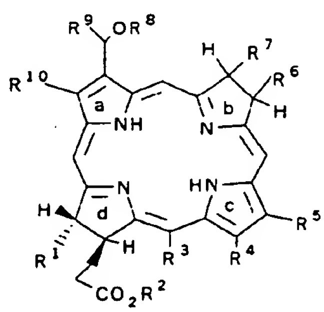
(71) Applicant: HEALTH RESEARCH, INC.
Buffalo New York 14263 (US)

(72) Inventors:
- Pandey, Ravindra K.
Williamsville, NY 14221 (US)
- Dougherty, Thomas J.
Grand Island, NY 14072 (US)
- Potter, William R.
Grand Island, NY 14072 (US)

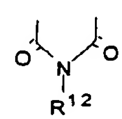
(74) Representative: Baldock, Sharon Claire et al
BOULT WADE TENNANT, Verulam Gardens 70
Gray's Inn Road
London WC1X 8BT (GB)

(54) **Long wavelength absorbing bacteriochlorin alkyl ether analogs**

(57) Novel compounds that either preferentially absorb into hyperproliferative tissue and absorb light efficiently at a wavelength of between about 700 and about 850 nm. or act as intermediates for such absorbing compounds. More particularly, the compounds of the invention have the formula:



where R^1, R^5, R^9, and R^{10} are independently lower alkyl of 1 to 3 carbon atoms provided that at least three of R^1, R^5, R^9, and R^{10} are methyl; R^2 is -OH, -OR^{11}, -NHR^{11}, aryl, or -aminoacid; R^3 and R^4 are independently -COR^{11} or taken together are

 ;

R^6 and R^7 are independently lower alkyl of 1 to 3 carbon atoms; R^8 is O-alkyl of 1 to about 12 carbon atoms, S-alkyl of 1 to about 12 carbon atoms, aryl, or a heterocyclic ring of 5 or 6 carbon atoms; R^{11} is alkyl of 1 to 6 carbon atoms; and R^{12} is lower alkyl of 1 to about 12 carbon atoms, aryl, or aminoalkyl of 1 to 8 carbon atoms; provided that at least one of R^8, R^{11}, and R^{12} is hydrophobic and together contain at least 10 carbon atoms. The invention also includes method of making and using the compounds.

EP 1 164 136 A1

Printed by Jouve, 75001 PARIS (FR)

Description

Background of the Invention

[0001] This invention relates to compounds for treatment and detection of hyperproliferative tissues such as tumors using photodynamic methods. These compounds have the ability to preferentially collect in such tissues when injected into an organism and that absorb light either to cause reduction in growth of the tissue, such as by its destruction or to cause emission of energy from the tissue that can be detected to locate the tissue. Such reduction and detection using photodynamic compounds is collectively referred to herein as photodynamic therapy.

[0002] Photodynamic therapy (PDT) is a relatively new modality for the treatment of various types of solid tumors. Many porphyrins and related photosensitive compounds demonstrate the ability to selectively accumulate in neoplastic tissue after intravenous injection and sensitize the tissue to photoirradiation. Activation of the photosensitive agent by visible light, delivered by a laser through fiber optics, results in the generation of cytotoxic agents. It is currently accepted that the production of singlet oxygen, formed from molecular oxygen, formed from molecular oxygen by the transfer of energy directly or indirectly from the activated photosensitizer, is responsible for tumor homeostasis and the observed tumor destruction.

[0003] Following absorption of light, the photosensitizer is transformed from its ground singlet state (P) into an electronically excited triplet state ($^3P*$; $\tau \sim 10^{-2}$ sec.) via a short-lived excited singlet state ($^1P*$; $\tau \sim 10^{-6}$ sec.) The excited triplet can undergo non-radiative decay or participate in an electron transfer process with biological substrates to form radicals and radical ions, which can produce singlet oxygen and superoxide ($O_2^{\cdot}$) after interaction with molecular oxygen (O_2). Singlet oxygen is the key agent responsible for cellular and tissue damage in PDT, causing oxidation of the target tissue (T); there also is evidence that superoxide ion may be involved.

[0004] In 1978, it was reported that a combination of hematoporphyrin derivative (HpD) and light was effective in causing partial or complete tumor necrosis in 111 of 113 tumors in 25 patients. PDT with Photofrin®, a purified HpD, has been approved in Canada for bladder and esophageal cancer; in the Netherlands and France for early and advanced stage esophageal cancer; in Japan for early stage lung, esophageal, gastric, and cervical cancer; and in the United States for advanced stage esophageal and lung cancers. More than 10,000 patients worldwide have been treated with PDT for a multiplicity of tumors accessible to light, including skin, lung, bladder, head and neck, breast, and esophageal cancers. Photofrin®, the current commercially used photosensitive drug, has some desirable characteristics, including good efficacy, water solubility, good yield of singlet oxygen, and ease of manufacture. However, Photofrin® has some disadvantageous properties: (i) it is a complex mixture of porphyrin dimers and higher oligomers linked by ether, ester, and/or carbon-carbon bonds and, therefore is difficult to study; (ii) it shows skin phototoxicity in patients for four to six weeks after administration; (iii) due to its relatively weak absorbance in the red region (630 nm), lack of penetration of light through tissue limits current clinical applications of Photofrin® in PDT to the destruction of cancerous tissue less than 4 mm from the source of light used in the therapy.

[0005] It has been established that both absorption and scattering of light by tissue increase as the wavelength decreases. Therefore, tissue penetration increases as the wavelength increases. Heme proteins in tissue account for most of the absorption of light in the visible region, and in tissue, light penetration drops off rapidly below 550 nm. However, there is a significant increase in penetration from 550 to 630 nm, and penetration doubles again to 700 nm. This is followed by a 10% increase in tissue penetration as the wavelength moves towards 800 nm.

[0006] Another reason that sets the ideal wavelength to 700-800 nm is the availability of the light sources in this region. Currently available laser lights used at 630 nm are expensive and not easy to handle clinically. A better solution is to use diode lasers. Advantages of diode lasers are low cost, negligible running cost, high reliability, small size and portability. Although diode lasers are now becoming available at 630 nm, photosensitizers with absorption between 700 to 800 nm in conduction with diode lasers are still desirable for treating tumors that are deeply seated. All these factors establish 700 to more than 800 nm as the optimal wavelength absorption for an efficient photosensitizer. Besides the properties discussed previously, the preferential tumor localization, stability, singlet oxygen producing efficiency, stability, low toxicity and solubility in appropriate injectable solvents are other important factors to be considered in developing an effective PDT agent.

[0007] In recent years, a number of long wavelength (>650 nm) absorbing photosensitizers have been reported as potential candidates for achieving maximum tissue penetration. Among such compounds, some naturally occurring bacteriochlorophylls have been reported as effective photosensitizers in preliminary *in vitro* and *in vivo* studies. However, most of the naturally occurring bacteriochlorins which have absorptions at 760-780 nm are extremely sensitive to oxidation, which results in a rapid transformation into the chlorin state which has an absorption maximum at or below 660 nm (see Fig. 1). Furthermore, if a laser is used to excite the bacteriochlorin *in vivo*, oxidation may result in the formation of a new chromophore absorbing outside the laser window, which reduces the photosensitizing efficacy. In order to render PDT more generally applicable to tumor therapy, there is need for long wavelength absorbing photosensitizers, such as, stable bacteriochlorins, which should also be able to localize in relatively high concentration at

the tumor site related to normal tissues.

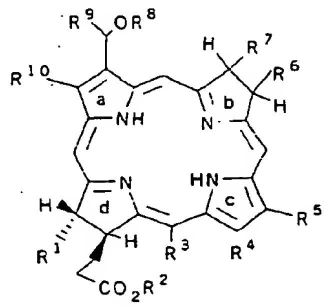
[0008] It is therefore an object of the invention to develop a stable photosensitizer that preferentially absorbs into hyperproliferative tissue and absorbs light efficiently at a wavelength of from about 700 to about 850 nm.

[0009] It is a further object of the invention to provide a method for photodynamic therapy using such stable photosensitizers.

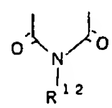
Brief Description of the Invention

[0010] In accordance with the invention novel compounds are therefore provided that either preferentially absorb into hyperproliferative tissue and absorb light efficiently at a wavelength of between about 700 and about 850 nm or act as intermediates for such absorbing compounds.

[0011] More particularly, the compounds of the invention have the formula:



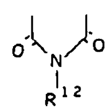
where R^1, R^5, R^9, and R^{10} are independently lower alkyl of 1 to 3 carbon atoms provided that at least three of R^1, R^5, R^9, and R^{10} are methyl; R^2 is -OH, -OR^{11}, -NHR^{11}, aryl, or -aminoacid; R^3 and R^4 are independently -COR^{11} or taken together are

 ;

R^6 and R^7 are independently lower alkyl of 1 to 3 carbon atoms; R^8 is O-alkyl of 1 to about 12 carbon atoms and usually 1 to 8 carbon atoms, S-alkyl of 1 to about 12 carbon atoms and usually 1 to 8 carbon atoms, aryl, or a heterocyclic ring of 5 or 6 carbon atoms; R^{11} is alkyl of 1 to 6 carbon atoms; and R^{12} is lower alkyl of 1 to about 12 carbon atoms, aryl, or aminoalkyl of 1 to 8 carbon atoms; provided that at least one of R^8, R^{11}, and R^{12} is hydrophobic and together contain at least 10 carbon atoms.

[0012] Especially suitable absorbing compounds of the invention have at least one pendant group sufficiently hydrophobic to cause the compound to enter hyperproliferative tissue. Such pendant group usually includes an aliphatic or aromatic structure containing at least two carbon atoms and, when acting as the primary hydrophobic moiety, usually contains at least seven carbon atoms. The compound may have more than one pendant hydrophobic group.

[0013] Examples of specific structures that are able to preferentially collect in hyperproliferative tissue are those compounds wherein R^2 is -OR^{11} and R^{11} is n-alkyl of 3 to about 10 carbon atoms, e.g. n-propyl; those compounds wherein R^3 and R^4 taken together are



where R^{12} is alkyl of 3 to about 10 carbon atoms, e.g. n-hexyl; and those compounds where R^8 is alkyl of 3 to about 10 carbon atoms, e.g. n-heptyl.

[0014] In preferred compounds of the invention, R^1, R^5, R^{7}, R^9, and R^{10} are all methyl and R^6 is ethyl.

[0015] The invention also includes the methods for treating and detecting hyperproliferative tissue such as tumors, by exposing the tissue to an amount of the absorbing compound of the invention which is effective for detecting or reducing the growth of the tissue upon exposure to sufficient light at a wave length between 700 and 850 nm.

[0016] In a preferred embodiment, the invention further includes facile approaches for the preparation of bacteriopurpurin-18-N-alkyl imides and their conversion into the corresponding 3-deacetyl-3-alkylether analogs with carboxylic acid, ester or amide functionalities and for the preparation of bacteriochlorin p_6 and its conversion into a series of alkyl ether analogs with carboxylic acid, ester or amide functionalities. The invention also includes use of these novel bacteriochlorins for the treatment of cancer or other non-oncological diseases by photodynamic therapy.

Detailed Description of the Invention

[0017] The compounds of the invention are unique in that they are bacteriochlorins, i.e. they have diagonally opposite fused reduced pyrrol rings (rings b and d) and have an alkyl ether group attached to the "a" fused pyrrol ring. The compounds of the invention have peak light absorbence at a wave length of between about 700 and about 850 nm and usually between 750 and 825 nm. The compounds further are uniquely stable due to the presence of an electron withdrawing group attached to the "c" fused pyrrol ring. The electron withdrawing group is preferably a stable six member fused imide ring or the radical $-COR_4$ where R^4 is -OH; -O-alkyl of 1 to about 10 carbon atoms; -NH-alkyl of 1 to about 12 carbon atoms; aryl, electron withdrawing at its position of attachment; or an amino acid radical.

[0018] The compounds of the invention suitable for injection into a mammal for preferential accumulation in hyperproliferative tissue also have at least one and preferably at least two pendant hydrophobic groups that assist in causing the compound to enter the hyperproliferative tissue.

[0019] "Hyperproliferative tissue" as used herein means tissue that grows out of control and includes tumors and unbridled vessel growth such as blood vessel growth found in age related macular degeneration.

[0020] In using compounds of the invention for photodynamic therapy, the compounds are usually injected into the mammal, e.g. human, to be diagnosed or treated. The level of injection is usually between about 0.1 and about 0.5 μmol/kg of body weight. In the case of treatment, the area to be treated is exposed to light at the desired wave length and energy, e.g. from about 100 to 200 J/cm^2. In the case of detection, fluorescence is determined upon exposure to light at the desired wave length. The energy used in detection is sufficient to cause fluorescence and is usually significantly lower than is required for treatment.

[0021] The invention includes a method for preparing compounds of the invention without requiring complex and inefficient synthesis steps.

[0022] For the preparation of bacteriopurpurin 1 (Fig. 2), the n-propyl alcohol extract of Rb Sphaeroides, which contains bacteriochlorophyll-a (λ_{max} 774 nm), was directly reacted with KOH/n-propanol in presence of air. It was immediately treated with HCl or H_2SO_4 (pH 2 to 3) to produce bacteriopurpurin-18 propyl ester and the related carboxylic acid 2 which in reacting with H_2SO_4/n-propanol can be converted into the related propyl ester analog 1. Compared to the naturally occurring bacteriochlorophyll-a, bacteriopurpurin with a fused anhydride ring system 2 (813 nm) was found to be extremely stable at room temperature. However, it was found to be unstable *in vivo.*

[0023] Compared to the anhydride ring system, compounds with fused imide ring system in other compounds have shown stability *in vivo.* For example, among non-porphyrin systems, amonafide, an imide derivative and its structural analogs are reported as anti-neoplastic agents *in vitro* as well as *in vivo* with good stability. While we could not know how this might apply to non-porphyrin systems, we investigated the effect of such cyclic structures in the bacteriochlorin system. Initially we followed our own methodology developed for the preparation of purpurin-18-N-alkylimides (U.S. Patent 5,952,366 incorporated herein by reference). Unfortunately, that approach produced a complex reaction mixture. Thus, in a modified approach, bacteriopurpurin-a 2 was first reacted with an alkyl amine (e.g. n-hexyl amine). the formation of the intermediate amide was monitored by spectrophotometry and analytical thin layer chromatography. The intermediate amide analog 3, obtained as a mixture of two isomers, was reacted with diazomethane and the solvent was removed under vacuum. The residue so obtained was re-dissolved in tetrahydrofuran and solvent was evaporated. This procedure was repeated several times until the disappearance of the absorption at 765 nm and appearance of a new peak at 822 nm. The bacteriochlorin-N-hexylimide so obtained had the required spectroscopic characteristic necessary for an "ideal" photosensitizer, and was stable *in vitro* and *in vivo,* but unfortunately did not produce any significant *in vivo* PDT efficacy.

[0024] Our next step was to investigate the effect of alkyl ether substitutions in bacteriochlorin series since similar substitutions in non-bacteriochlorin systems sometimes enhanced tumor localization see e.g. U.S. Patents 5,459,159 and 5,952,366 both of which are incorporated herein by reference. In order to introduce various alkyl ether substituent at the peripheral position, the bacteriopurpurinimide 4 containing an acetyl group at position 3 was first reduced to the corresponding 3-(1-hydroxyethyl) 5 by reacting with sodiumborohydride in excellent yield, which on dehydration by refluxing in o-dichlorobenzene for 5 min produced the vinyl analog 6 in >80% yield. For the preparation of the desired alkyl ether analog, the hydroxy analog 6 was treated with HBr/acetic acid, and the intermediate bromo- derivative was

immediately reacted with various alkyl alcohols, and the corresponding alkyl ether analogs (e.g. 7) were isolated in about 70% yield. Under similar reaction conditions, the vinyl bacteriopurpurin-imide also produced the desired alkyl ether derivatives, but in low yield (Fig. 2).

[0025] This invention also deals with the synthesis of the alkyl ether analogs of bacteriopurpurin p_6 and their amide derivatives (λ_{max} 760 nm). For the preparation of these compounds, bacteriopurpurin-18 methyl ester 7 was reacted with aqueous sodium carbonate or sodiumhydroxide/THF solution. The dicarboxylic derivative 8 obtained by the cleavage of the fused anhydride ring system was converted into the corresponding methyl ester 9 upon reacting with diazomethane. Reaction of 9 with sodiumborohydride and subsequent treatment with HBr/acetic acid and various alkyl alcohols will generate the desired alkyl ether derivatives (Fig. 3). The regiospecic hydrolysis of the propionic ester functionality into the corresponding carboxylic acid and subsequent conversion into various amides could generate a series of amide analogs (see Fig. 4).

[0026] The following examples serve to illustrate and not limit the present invention: melting points are uncorrected and were measured on a Fisher Johns melting point apparatus. Electronic absorption spectra were measured on a Genesis 5 spectrophotometer. Mass spectra were measured at the Department of Molecular and Cellular Biophysics, RPCI, Buffalo. NMR spectra were obtained at 400 MHz Brucker instrument at the NMR facility of the institute. Samples were dissolved in $CDCl_3$ and the chemical shifts are expressed in δ ppm relative to $CHCl_3$ at 7.258 ppm. Analytical thin layer chromatography was used to monitor the reactions and to check the purity of the desired compounds on cut strips of Merck or Whatman silica gel 60F254 precoated (0.25 mm thickness) plastic backed sheets. For column chromatography Silica gel (70-230 mesh) was used for normal gravity column.

[0027] Tetrahydrofuran (THF) was distilled over sodium and dichloromethane over calcium hydride before use. The phase dried, filtered and evaporated means drying over sodium sulfate, filtering through glass wool, and then evaporating off the solvent using a Buchi rotary evaporator under house vacuum or high vacuum achieved with an oil pump.

Example 1 - Preparation of 3-acetyl-bacteriopurpurin-18-propyl ester 1

[0028] *Rb sphaeroides* (350 gram) was dissolved in ether (200 ml) and pyridine (10 ml). Sodium hydroxide (12g) dissolved in n-propanol (100 ml) was added and a stream of air was bubbled through the solution with constant stirring for 2h. The ethereal layer was removed, and the pH of the aqueous phase was adjusted by adding H_2SO_4 to 2.5. The solvent was removed under vacuum. The residue so obtained was redissolved in THF and evaporated. This process was repeated several times till the peak at 765 disappeared and a new peak appeared at 804 nm. After removing the solvent the residue was found to be a mixture of two compounds and separated by column chromatography. The faster moving band was identified as the title product, whereas the slower moving band was characterized as the related carboxylic acid analog, which on treating with 5% sulfuric acid/n-propanol produced the corresponding propyl ester. Yield: 250 mg.

Example 2 - Preparation of 3-Acetyl-bacteriopurpurin-18-N-hexylimide 4

[0029] Bacteriopurpurin-18 propyl ester 1 (200 mg) was dissolved in dichloromethane (10 ml) and n-hexylamine (0.5 ml) was added. The reaction was stirred at room temperature for overnight. The reaction was monitored by TLC and spectrophotometry (disappearance of a peak at 804 nm and appearance of a new peak at 765 nm). The solvent was removed under high vacuum and the residue was dissolved in dichloromethane. It was then treated with diazomethane to convert the carboxylic acid functionality into the corresponding methyl ester. THF was then added and solvent was removed under vacuum till the intensity of the amide peak at 760 reduced to 10% and a new peak caused by the formation of the title compound appeared at 822 nm. It was then purified by silica column chromatography using 2% acetone/dichloromethane as eluent. The residue obtained after evaporating the solvent was precipitated with dichloromethane/hexane mixture. Yield: 112 mg. NMR (δ ppm, $CDCl_3$): 9.31 (s, 1H, 5-H); 8.80 (s, 1H, 20-HO); 5.29 (d, 1H, 17-H); 4.42 (t, 2H, hexylimide-a-CH_2); 4.29 (m, H, 3-H); 4.09 (m, 3H, CO_2CH_2 and 18-H); 3.94 (m, 2H, 7-H and 8-H); 3.70 (s, 3H, 12-Me); 3.55 (s, 3H, 2-Me); 3.17 (s, 3H, 3-Me); 2.68 (M, 1H, 17b-H); 2.41 (m, 5H, $CH_2CH_2CH_3$ + 8a-CH_2 + 7b'H); 2.04 (m, 4H, 17a-H, 17a'-H and b, c-N-hexyl-CH_2); 1.70, 1.67 (each d, 3H, 18-Me and 7-Me); 1.32 (m, 4H, d, e-hexylimide-CH_2); 1.14 (t, 3H, 3-b Me); 0.93 (t, 3H, $CH_2CH_2CH_3$); -0.53 and -0.75 (each br s, 2H, 2NH). Mass calculated for $C_{42}H_{53}N_5O_5$: 707. Found: 707.9 (M + 1). Long wavelength absorption λ_{max} 822 nm.

Example 3 - Preparation of 3-Deacetyl-3-(1-hydroxyethyl)bacteriopurpurin-18-N-hexylimide 5

[0030] The foregoing bacteriopurpurin-imide 4 (100 mg) was dissolved in dichloromethane (10 ml) and methanol (5 ml). Sodium borohydride (30 mg) was added slowly (within 30 min) with continuous stirring at 0°C. The reaction was monitored by TLC and spectrophotometry (appearance of a new peak at 786 nm). It was then diluted with dichloromethane. The organic layer was washed with 5% acetic acid and again with water. It was dried over sodium sulfate. Evap-

oration of the solvent gave the desired product, 80 mg. NMR (δ ppm, $CDCl_3$): 8.81 (d, 1H, 5-H); 8.00 (s, 1H, 20-H); 8.25 (d, 1H, 17-H); 6.18 (q, 1H, $CH(OH)CH_3$); 4.42 (t, 2H, hexylimide-a-CH_2); 4.29 (m, H, 3-H); 3.94 (m, 7H and 8-H); 3.82 (m, 3H, CO_2CH_2 and 18-H); 3.60 and 3.20 (each s, 3H, 3-Me); 2.68 (m, 1H, 17b-H); 2.41 (m, 5H, $CH_2CH_2CH_3$ + 8a-CH_2 + 7b'H); 2.04 (m, 4H, 17a-H, 17a'H and b, c-N-hexyl-CH_2); 2.10 (d, 3H, 18-Me); 1.80 (m, 2H, 8-CH_2CH_3) and 1.75 - 1.30 (m, 4H, d,e-hexylimide-CH_2); 1.10. 0.93 and 0.759 (total 9H: t, 3H, 3-b Me), (t, 3H, $CH_2CH_2CH_3$); -0.03 and -0.45 (each br s, 2H, 2NH). Mass calculated for $C_{42}H_{55}N_5O_5$: 709. Found: 709.9 (M + 1). Long wavelength absorption λ_{max} 786 nm.

Example 4 - Preparation of 3-Deacetyl-3-vinyl-bacteriopurpurin-18-N-hexylimide propylester 6

[0031] The hydroxy analog 5 (20 mg) was added to refluxing o-dichlorobenzene (5 ml) and the solution was stirred for 5 min. It was then cooled to room temperature. The solution was passed through a silica column, eluted first with hexane to remove theo-dichlorobenzene and then with 2% acetone in dichloromethane. Evaporation of the major band gave a residue, which was crystallization from dichloromethane/hexane in 70% yield. NMR (δ ppm, $CDCl_3$): 8.61 (d, 1H, 5-H); 8.42 (s, 1H, 20-H); 8.38 (d, 1H, 17-H); 7.75 (m, 1H, CH=CH_2); 6.18, 6.08 (each d, 1H, CH=CH_2); 4.42 (t, 2H, hexylimide-a-CH_2); 4.29 (m, H, 3-H); 3.94 (m, 2H, 7-H and 8-H); 3.82 (m, 3H, CO_2CH_2 and 18-H); 3.60 (s, 3H, 3-Me); 3.22 (s, 3H, CH_3); 2.62 (m, 1H, 17b-H); 2.31 (m, 5H, $CH_2CH_2CH_3$ + 8a-CH_2 + 7b'H); 2.04 (m, 4H, 17a-H, 17a'H and b, c-N-hexyl-CH_2); 1.78 and 1.62 (each d, 3H, 18-Me and 7-Me); 1.80 (m, 2H, 8-CH_2CH_3) and 1.65 - 1.30 (m, 4H, d, e-hexylimide-CH_2); 1.10. 0.93 and 0.80 (total 9H: t, 3H, 3-b Me), (t, 3H, $CH_2CH_2CH_3$); -0.03 and -0.40 (each brs, 2H, 2NH). Mass calculated for $C_{42}H_{53}N_5O_4$: 691. Found: 691.7 (M + 1). Long wavelength absorption λ_{max} 788 nm.

Example 5 - Preparation of 3-Deacetyl-3-(1-heptyloxyethyl)-bacteriopurpurin-N-hexylimide propyl ester 7

[0032] The foregoing bacteriopurpurin 6 (30 mg) was reacted with 30% HBr/acetic acid (1.5 ml) in room temperature for 2h. The solvents were removed under high vacuum. The residue so obtained was dissolved in dry dichloromethane (5 ml) and immediately reacted with n-heptanol (1 ml). A small amount of anhydrous potassium carbonate was added before leaving the reaction at room temperature under an inert atmosphere for 45 min. It was then diluted with dichloromethane. After the standard work-up, the residue was purified by silica column chromatography. Yield 20 mg. NMR (δ ppm, $CDCl_3$): 8.82 (d, 1H, 5-H); 8.62 (s, 1H, 20-H); 8.30 (d, 1H, 17-H); 5.60 (q, 1H, CH(O-heptyl)CH_3); 5.25 (m, H, 17-H); 4.42 (t, 2H, hexylimide-a-CH_2); 4.20 (m, 3H, CO_2CH_2 and 18-H); 3.94 (m, 2H, 7-H and 8-H); 3.80 (m, O-CH_2 of heptyl ether chain); 3.65 (s, 3H, 3-Me); 3.25 (s, 3H, CH_3); 2.62 (m, 1H, 17b-H); 2.31 (m, 5H, $CH_2CH_2CH_3$ + 8a-CH_2 + 7b'H); 2.00-0.75, several multiplets: (m, 4H, 17a-H, 17a'H and b, c-N-hexyl-CH_2); 1.80 and 1.52 (each d, 3H, 18-Me and 7-Me); 1.80 (m, 2H, 8-CH_2CH_3) and 1.65 - 1.30 (m, 4H, d,e-hexylimide-CH_2 and 8H of the O-heptyl side chain); 1.10. 0.93 and 0.80 (total 12H: t, 3H, 3-b Me and O-heptyl-Me) and (t, 3H, $CH_2CH_2CH_3$); -0.03 and -0.40 (each brs, 2H, 2NH). Mass calculated for $C_{49}H_{69}N_5O_5$: Calculated: 807. Found: 808.3 (M + 1). Long wavelength absorption λ_{max} 786 nm.

[0033] The title compound was also obtained from the vinyl analog 6 by following the same methodology. However, the desired product was obtained in low yield.

Example 6 - Preparation of Bacteriopurpurin p_6 trimethyl ester

[0034] Bacteriopurpurin-18-methylester (50 mg) was dissolved in anhydrous THF (20 ML). Aqueous solution of sodium hydroxide or sodium carbonate was added. The reaction was stirred at room temperature till the parent peak at 804 nm disappeared. the pH was then slowly adjusted to 5, extracted with dichloromethane/THF mixture. The organic layer was washed with water, dried over anhydrous sodium sulfate, and the solvent was evaporated. The residue was converted into the corresponding methyl ester by reacting diazomethane, and purified by column chromatography (Silica gel). Yield 40 mg. NMR (δ ppm, $CDCl_3$): 9.70, 8.72, 8.60 (each s, 1H, 3-meso H); 5.00 (d, 1H, 17-H); 4.20 (m, 1H, 18-H); 3.95 (m, 2H, 7-H and 8-H); 4.12, 4.10, 3.60, 3.58, 3.50, 3.20 (each s, 3H, 3 CO_2Me, 2Me and CO_2Me); 2.50 - 2.00 (m, 6H, 12-$CH_2CH_2CO_2Me$ and 8-CH_2CH_3); 1.80 and 1.70 (each d, 3H, 7-Me and 18-Me); 1.20 (t, 3H, CH_2Me); -90 and -85 (each s, 1H, 2NH). Found: Long wavelength absorption λ_{max} 760 nm.

Example 7 - Biological Studies

[0035] The photosensitizers were dissolved in known quantity of Tween 80 (Aldrich) surfactant and diluted by a factor of 10 with 5% dextrose solution in water to produce a final Tween 80 concentration of 1%. The solution was then filtered through a syringe filter. The concentration of the solution was determined on the basis of the extinction coefficient value of the photosensitizer at the longest wavelength absorption.

[0036] Before injecting the drug to the animals, the purity of the material was confirmed by HPLC and it was performed

using a Spectra-Physics system connected to a SP8 700 solvent delivery system, Kratos 757 absorption detector with a fixed wavelength ant 405 or 786 nm. Two solvent systems were used in the HPLC analysis: solvent A was prepared by dissolving anhydrous dibasic sodium phosphate (1.0 g) in 400 ml water. To this was added HPLC grade methanol (600 ml). The pH of the solution was adjusted to 7.5 with phosphoric acid; and (ii) solvent B was prepared by dissolving anhydrous dibasic sodium phosphate (0.3 g) in 100 ml water, and to this was added methanol (900 ml) and the pH was adjusted to 7.5 with phosphoric acid. Solvents A and B were used as gradient mode (0 - 10 min A, 10 - 40 min A - B, 40 - 50 min B, 50 - 60 min back to A). In some cases solvent B was used as isocratic mode (column reverse phase C-8, flow rate 1.5 ml/min). Prior to irradiation, the fur over grown and surrounding the tumor was removed with electric clippers. Twenty four hours after injecting the drug, the mouse was placed in a custom made aluminum holder. Standard light dose 75mW/cm^2 for 30 min for a total incident light dose of 135J/cm^2 from a tunable dye laser tuned to the maximum red absorption peak at 790 nm (*in vivo* absorption, determined by *in vivo* reflectance spectroscopy). Laser output was measured with a power meter.

[0037] Following light exposure, the mice were kept in groups of 5/cage and supplied with pelleted food and water ad libitum. Tumor size and gross appearance of both tumor and overlying skin was monitored daily for 90 days after photo-illumination unless growth of non-responsive tumor require early sacrifice of those animals.

[0038] Bacteriopurpurin-imides 5 - 7 above have been evaluated for *in vivo* studies in a mouse tumor model system (RIF tumor). Results are summarized in Table 1. From these results it can be seen that among the compounds tested, 3-deacetyl-3-(1-heptyloxyethyl) purpurinimide-18 7 produced significant photosensitizing activity at a dose of 0.47 μmol/kg. The mice were treated with light (790 nm, 135 J/cm^2) after 24h post injection of the drug (80% tumor cure on day 21 and 60% on day 90). At a higher drug dose (1.0 μmol/kg), all mice died (6/6) after the light treatment, suggesting that the drug is quite potent. The efficacy of the drug was also determined at variable drug and light doses. For example, reducing the drug dose to 0.2 μmol/kg and keeping the same light dose (135 J/cm^2) did not show any PDT efficacy, however, at the higher light dose (175 J/cm^2) four out of six mice were tumor free on day 90. Under similar treatment conditions bacteriochlorins 5 and 6 did not produce any PDT efficacy.

TABLE 1

In vivo photosensitizing efficacy of bacteriopurpurinimides against RIF rumor (C_3H mice)

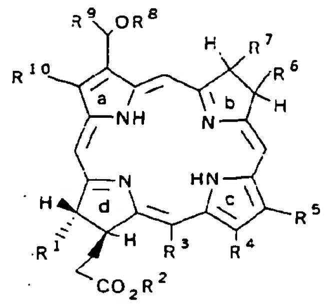
Compound No.	Injected Dose (μmol/kg)	Light Dose (790 nm) 24h post injection	Tumor Response (%)		
			Day 7	Day 21	Day 90
7	1.00	135J/cm^2	ALL MICE DIED		
	0.47	135J/cm^2	80	80	60
	0.2	135J/cm^2	NO RESPONSE		
	0.2	175J/cm^2	100	70	70
5	1.0	135J/cm^2	NO RESPONSE		
6	1.0	135J/cm^2	NO RESPONSE		

[0039] The tumor uptake and *in vivo* shift in the long wavelength absorption of the bacteriopurpurin-imide 7 was determined by *in vivo* reflectance spectroscopy. Bacteriopurpurinimide 7 had significantly higher tumor uptake at day 5 than day 1 post injection of the drug. Compared to *in vitro* absorption, the long wavelength absorption *in vivo* was observed at 790 nm, exhibiting a red shift of about 5 nm. Thus, the tumors were irradiated with light at that particular wavelength. This experiment also suggests that the fused imide ring system is quite stable *in vivo* even after 5 day post injection of the photosensitizer. *In vivo* studies with these and other bacteriochlorin analogs at variable treatment conditions are currently in progress.

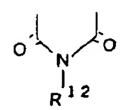
[0040] Since prolonged cutaneous photosensitivity is a serious side-effect of Photofrin® administration, we tested the phototoxicity of 3-deacetyl-3-(1-heptyloxyethyl) bacteriopurpurin-18-N-hexylimide 7 in mouse foot tissue and the therapeutic drug and light doses. Mice were injected (I.V.) with 0.47 mmol/kg of the drug. Feet were illuminated with 135J/cm^2 at 790 nm laser light on days 1, 2, 3, 4 and 5 (Fig. 5). Foot response was graded according to the following arbitrary scale: 0, no difference from normal; 0.1, very slight edema; 0.3, slight edema; 0.5, moderate edema; 0.75, large edema; 1, large edema with exudate; 1.2, moderate reddening with slight scaly or crusty appearance; 1.65, slight damage to toes; 1.75, definite damage or slight fusion of toes; 2.0, most toes fused; 2.5, foot almost shapeless with no toes; 3, only stub of foot remaining. As can be seen from Fig. 2, bacteriopurpurin-imide 7 did not show any toxicity when feet were illuminated 5 days after injection. These results suggest a possibility that this compound is cleared rapidly from mouse foot tissues, unlike Photofrin®, which showed a long term cutaneous phototoxicity.

Claims

1. A cyclic tetrapyrolle compound **characterized in that** it has the formula:



where R^1, R^5, R^9, and R^{10} are independently lower alkyl of 1 to 3 carbon atoms provided that at least three of R^1, R^5, R^9, and R^{10} are methyl; R^2 is -OH, -OR^{11}, -NHR^{11}, aryl, or -aminoacid; R^3 and R^4 are independently -COR^{11} or taken together are

 ;

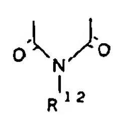
R^6 and R^7 are independently lower alkyl of 1 to 3 carbon atoms; R^8 is O-alkyl of 1 to about 12 carbon atoms, S-alkyl of 1 to about 12 carbon atoms, aryl, or a heterocyclic ring of 5 or 6 carbon atoms; R^{11} is alkyl of 1 to 6 carbon atoms; and R^{12} is lower alkyl of 1 to about 12 carbon atoms, aryl, or aminoalkyl of 1 to 8 carbon atoms; provided that at least one of R^8, R^{11}, and R^{12} is hydrophobic and together contain at least 10 carbon atoms.

2. The compound of claim 1 **characterized in that** the compound has a peak light absorption at a light wave length of between about 750 and 850 nm.

3. The compound of claim 2 **characterized in that** R^1, R^5, R^9, and R^{10} are all methyl.

4. The compound of claim 3 **characterized in that** R^2 is -OR^{11} and R^{11} is n-propyl.

5. The compound of claim 4 **characterized in that** R^3 and R^4 taken together are



and R^{12} is hexyl.

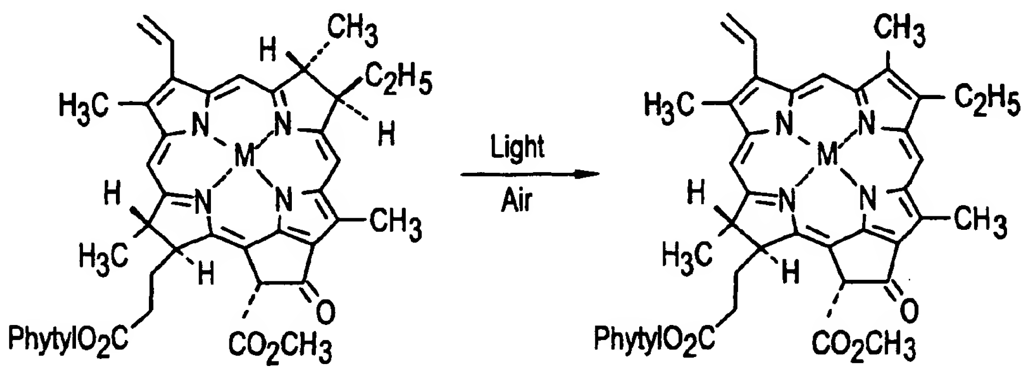
6. The compound of claim 5 **characterized in that** R^6 is ethyl and R^7 is methyl.

7. The compound of claim 6 **characterized in that** R^8 is heptyl.

8. The use of the compound of claims 2 through 7 **characterized in that** the use is for treating hyperproliferative tissue by exposing the tissue to a sufficient quantity of the compound to reduce growth of the tissue upon exposure to light at the peak absorption wave length.

9. The use of the compound of claims 2 through 7 **characterized in that** the use is for detecting the presence of hyperproliferative tissue by exposing the tissue to a sufficient quantity of the compound to cause a detectable light emission from the tissue, at a wave length different from the peak absorption wave length, upon exposure of the tissue to light at the peak absorption wave length.

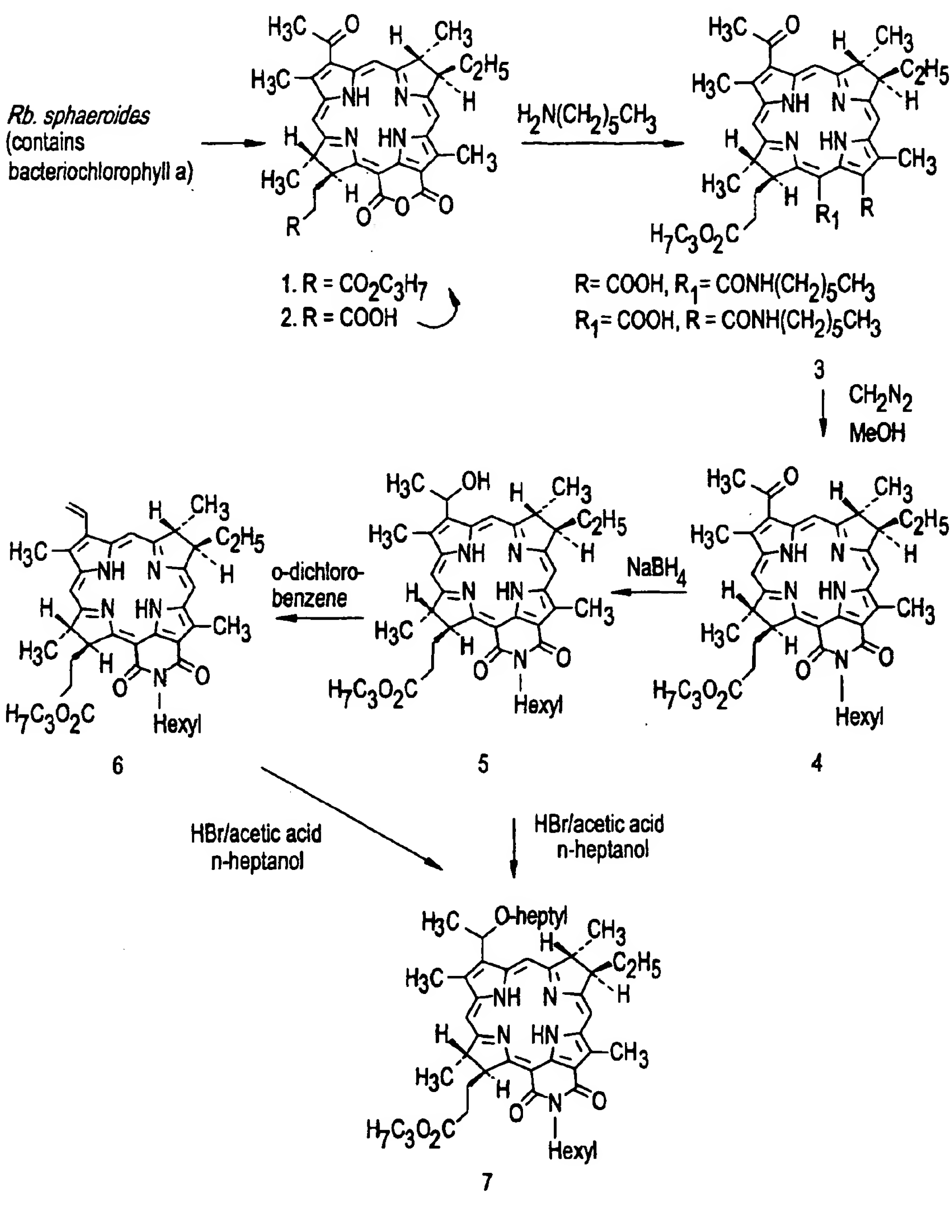
FIG. 1



Bacteriochlorin
(780 nm)

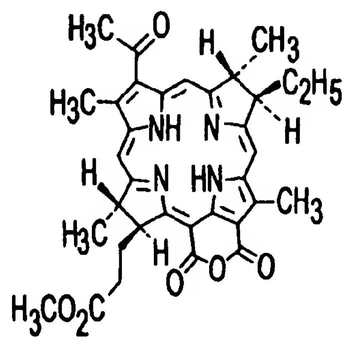
Chlorin
(660 nm)

FIG. 2

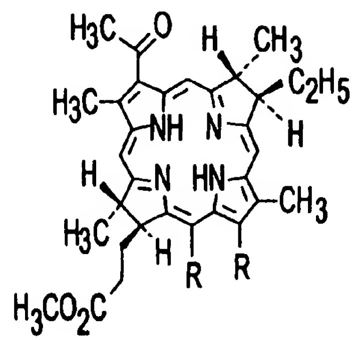


Rb. sphaeroides
(contains
bacteriochlorophyll a)
$H_2N(CH_2)_5CH_3$
1. R = $CO_2C_3H_7$
2. R = COOH
R= COOH, R_1= $CONH(CH_2)_5CH_3$
R_1= COOH, R = $CONH(CH_2)_5CH_3$
3
CH_2N_2
MeOH
4
$NaBH_4$
5
o-dichloro-
benzene
6
HBr/acetic acid
n-heptanol
HBr/acetic acid
n-heptanol
7

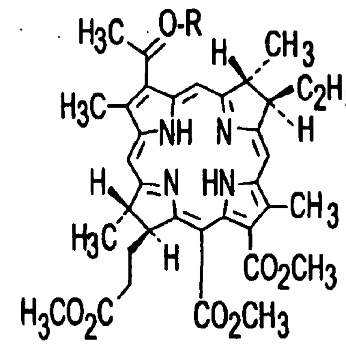
FIG. 3



8.
Bacteriopurpurin-18 methyl ester



9. R = COOH
10. R = CO_2CH_3



11.
R = Various alkyl groups

FIG. 4A

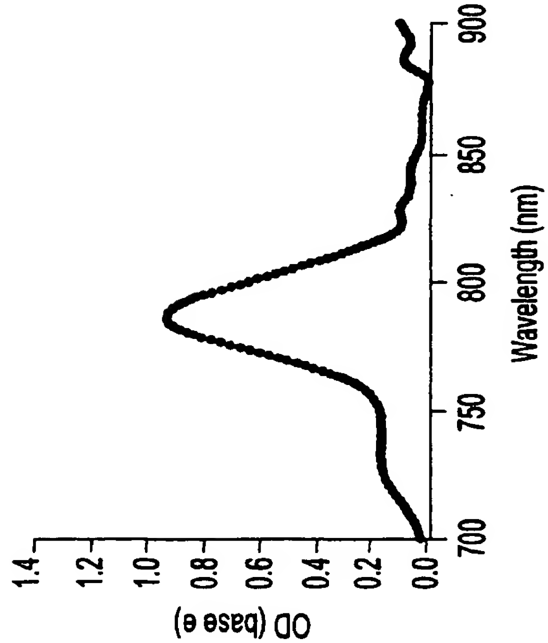

OD (base e)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
700
750
800
850
900
Wavelength (nm)

FIG. 4B

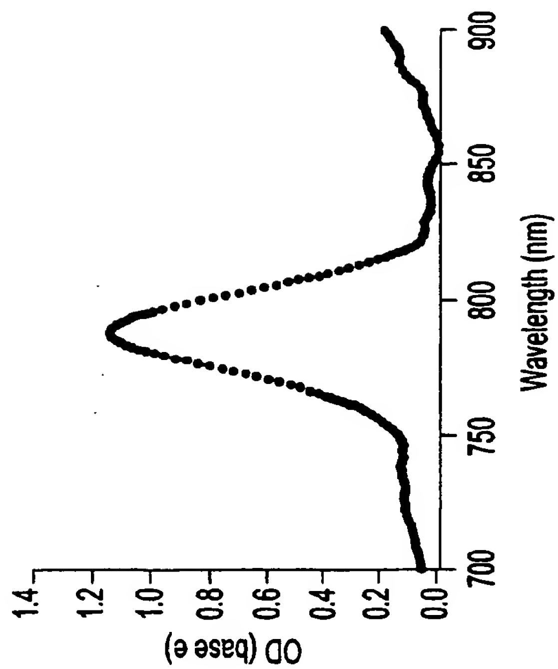
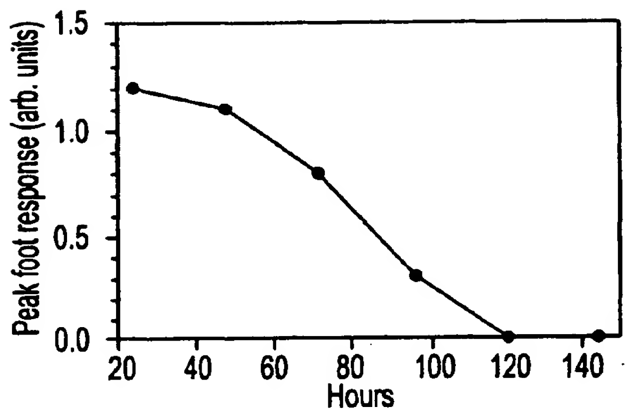

OD (base e)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
700
750
800
850
900
Wavelength (nm)

FIG. 5


Peak foot response (arb. units)
1.5
1.0
0.5
0.0
20
40
60
80
100
120
140
Hours

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application Number
EP 01 10 8984

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
Y	WO 95 32206 A (HEALTH RESEARCH INC ;UNIV CALIFORNIA (US)) 30 November 1995 (1995-11-30) * claims *	1-9	C07D487/22 C07D471/22 A61K41/00 A61P35/00 //(C07D487/22, 257:00,209:00, 209:00,209:00, 209:00), (C07D471/22, 257:00,221:00, 209:00,209:00, 209:00,209:00)
Y	WO 97 32885 A (HEALTH RESEARCH INC) 12 September 1997 (1997-09-12) * claims *	1-9	
D	& US 5 952 366 A 14 September 1999 (1999-09-14)		
Y	WO 93 13769 A (HEALTH RESEARCH INC) 22 July 1993 (1993-07-22) * claims *	1-9	
D	& US 5 459 159 A 17 October 1995 (1995-10-17)		
A	EP 0 276 121 A (UNIV BRITISH COLUMBIA) 27 July 1988 (1988-07-27) * claims *	1-9	TECHNICAL FIELDS SEARCHED (Int.Cl.7)
A	EP 0 891 977 A (DLR EV) 20 January 1999 (1999-01-20) * claims *	1-9	C07D A61K A61P
	-/--		

INCOMPLETE SEARCH

The Search Division considers that the present application, or one or more of its claims, does/do not comply with the EPC to such an extent that a meaningful search into the state of the art cannot be carried out, or can only be carried out partially, for these claims.

Claims searched completely :

Claims searched incompletely :

Claims not searched :

Reason for the limitation of the search:

Although claims 8,9 are directed to a method of treatment of the human/animal body (Article 52(4) EPC), the search has been carried out and based on the alleged effects of the compound/composition.

Place of search	Date of completion of the search	Examiner
THE HAGUE	3 September 2001	Chouly, J

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 01 10 8984

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
A	WO 96 17844 A (UNIV PENNSYLVANIA ;DIMAGNO STEPHEN (US)) 13 June 1996 (1996-06-13) * claims * ---	1-9	
A	WO 99 59641 A (UNIV ARIZONA ;MALLINCKRODT INC (US)) 25 November 1999 (1999-11-25) * claims * ---	1-9	
P,A	WO 00 61584 A (UNIV BRITISH COLUMBIA ;DOLPHIN DAVID (CA); MACALPINE JILL (CA); BR) 19 October 2000 (2000-10-19) * claims * ---	1-9	
P,A	WO 00 61585 A (STERNBERG ETHAN D ;UNIV BRITISH COLUMBIA UBC (CA); DOLPHIN DAVID () 19 October 2000 (2000-10-19) * claims * -----	1-9	TECHNICAL FIELDS SEARCHED (Int.Cl.7)

EPO FORM 1503 03.82 (P04C10)

ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO. EP 01 10 8984

This annex lists the patent family membersrelating to the patent documents cited in the above-mentioned European search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

03-09-2001

Patent document cited in search report		Publication date	Patent family member(s)			Publication date
WO 9532206	A	30-11-1995	US	5591847	A	07-01-1997
WO 9732885	A	12-09-1997	US	5770730	A	23-06-1998
			US	5952366	A	14-09-1999
			US	5864035	A	26-01-1999
WO 9313769	A	22-07-1993	US	5198460	A	30-03-1993
			AU	3433793	A	03-08-1993
			CA	2125189	A	22-07-1993
			EP	0623020	A	09-11-1994
			FI	942878	A	16-06-1994
			JP	7505614	T	22-06-1995
			NO	942283	A	16-06-1994
			SK	76894	A	12-04-1995
			US	5459159	A	17-10-1995
			US	5314905	A	24-05-1994
EP 0276121	A	27-07-1988	US	4883790	A	28-11-1989
			AT	104859	T	15-05-1994
			AU	618725	B	09-01-1992
			AU	1038888	A	21-07-1988
			CA	1333442	A	06-12-1994
			DE	3889231	D	01-06-1994
			DE	3889231	T	11-08-1994
			ES	2055735	T	01-09-1994
			JP	1999536	C	08-12-1995
			JP	6008319	B	02-02-1994
			JP	63277700	A	15-11-1988
			JP	2835294	B	14-12-1998
			JP	7258262	A	09-10-1995
			MX	9203250	A	31-07-1992
			US	5399583	A	21-03-1995
			US	5095030	A	10-03-1992
			US	5171749	A	15-12-1992
			US	5283255	A	01-02-1994
			US	4920143	A	24-04-1990
EP 0891977	A	20-01-1999	DE	19730469	A	21-01-1999
			DE	19743903	A	15-04-1999
			US	6107480	A	22-08-2000
WO 9617844	A	13-06-1996	US	5817830	A	06-10-1998
			CA	2208148	A	13-06-1996
			EP	0821686	A	04-02-1998
			JP	10512237	T	24-11-1998
			US	5955603	A	21-09-1999

EPO FORM P0459

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

ANNEX TO THE EUROPEAN SEARCH REPORT ON EUROPEAN PATENT APPLICATION NO. EP 01 10 8984

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

03-09-2001

Patent document cited in search report		Publication date	Patent family member(s)			Publication date
WO 9959641	A	25-11-1999	US	6183727	B	06-02-2001
			US	6207821	B	27-03-2001
			US	6274729	B	14-08-2001
WO 0061584	A	19-10-2000	AU	4139600	A	14-11-2000
			AU	4278400	A	14-11-2000
			WO	0061585	A	19-10-2000
WO 0061585	A	19-10-2000	AU	4139600	A	14-11-2000
			AU	4278400	A	14-11-2000
			WO	0061584	A	19-10-2000

EPO FORM P0459

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82